PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : A61M 1/36, A61K 35/14, 41/00

A1

(11) International Publication Number: WO 93/15778

(43) International Publication Date: 19 August 1993 (19.08.93)

(21) International Application Number: PCT/GB93/00258

(22) International Filing Date: 8 February 1993 (08.02.93)

(30) Priority data:
07/832,798 7 February 1992 (07.02.92) US

(71)(72) Applicant and Inventor: BOLTON, Anthony, Ernest [GB/GB]; 12 Thornsett Road, Sheffield S71 NB (GB).

(74) Agent: FRANKLAND, Nigel, H.; Forrester Ketley & Co., Forrester House, 52 Bounds Green Road, London N11 2EY (GB).

(81) Designated States: AU, CA, JP, KR, NZ, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: METHOD OF INHIBITING THE AGGREGATION OF BLOOD PLATELETS

(57) Abstract

A method of treating blood which comprises contacting blood with a blood platelet-aggregation inhibiting effect of amount of ozone gas and ultraviolet radiation. The blood may be administered to a patient such as a human for inhibiting the aggregation of blood platelets.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SK	Slovak Republic
CI	Côte d'Ivoire	KZ	Kazakhstan	SN	Senegal
CM	Cameroon	LI	Liechtenstein	SU	Soviet Union
CS	Czechoslovakia	LK	Sri Lanka	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	MC	Monaco	UA	Ukraine
DK	Denmark	MG	Madagascar	US	United States of America
ES	Spain	ML	Mali	VN	Viet Nam
FI	Finland	MN	Mongolia		

METHOD OF INHIBITING THE AGGREGATION OF BLOOD PLATELETS

Platelets are the smallest of the formed elements of the blood. Every cubic millimeter of blood contains about 250 million platelets, as compared with only a few thousand white cells. There are about a trillion platelets in the blood of an average human adult. Platelets are not cells, but are fragments of the giant bone-marrow cells called megakaryocytes. When a megakaryocyte matures, its cytoplasm breaks up, forming several thousand platelets. Platelets lack DNA and have little ability to synthesize proteins. When released into the blood, they circulate and die in about ten days. However, platelets do possess an active metabolism to supply their energy needs.

Because platelets contain a generous amount of contractile protein (actomyosin), they are prone to contract much as muscles do. This phenomenon explains the shrinkage of a fresh blood clot after it stands for only a few minutes. The shrinkage plays a role in forming a hemostatic plug when a blood vessel is cut. The primary function of platelets is that of forming blood clots. When a wound occurs, platelets are attracted to the site where they

activate a substance (thrombin) which starts the clotting process. Thrombin, in addition to converting fibrinogen into fibrin, also makes the platelets sticky. Thus, when exposed to collagen and thrombin, the platelets aggregate to form a plug in the hole of an injured blood vessel.

Platelets not only tend to stick to one another, but to the walls of blood vessels as well. Because they promote clotting, platelets have a key role in the formation of thrombi. The dangerous consequences of thrombi are evident in many cardiovascular and cerebrovascular disorders.

In this regard, the precise function of blood platelets in various human disease states has recently become increasingly understood as advances in biochemistry permit the etiologies of diseases to be better understood.

For example, many attempts have been made to explain the process of atherogenesis, that is, the creation of plaque which narrows arteries and, of particular concern, the coronary arteries. Recently, there has been increasing interest in the possible role of platelets in atherosclerosis.

In addition, a number of disease states in humans are believed to be associated with an aggregation of platelets in the blood. These platelet aggregation associated conditions include: peripheral vascular disease; thrombotic diseases such as coronary thrombosis and pulmonary thrombosis; stroke; eclampsia and pre-eclampsia; and hypertension.

A study completed by the University of Oxford, England, and published in the British Medical Journal, Vol. 296, January 30 1988, pages 320-331, entitled "Secondary Prevention of Vascular Disease by Prolonged Antiplatelet Treatment", suggests that therapies which inhibit platelet aggregation may be useful for treating occlusive vascular disease. The study utilised aspirin, sulphinpyrazone, or aspirin and dipyridamole as the platelet aggregation inhibiting agents.

Unfortunately, long-term aspirin therapy may lead to severe gastrointestinal irritation and bleeding. Also, these and other known agents which inhibit platelet aggregation may have other undesirable side-effects that make them unsuitable for administration to patients who could benefit from such therapy. For pregnant women with pre-eclampsia or other platelet aggregation associated conditions, the administration of drugs may be undesirable in view of the potential effects of the same on the developing fetus.

According to this invention there is provided a method of treating blood which comprises contacting the blood with a blood platelet-aggregation inhibiting effective amount of ozone gas and ultraviolet radiation.

Preferably the ozone gas has a concentration of from about 0.5 to about 100 μg/ml.

Advantageously the ozone gas has a concentration of from about 5 to about 50 μg/ml.

Conveniently the ultraviolet radiation has a wavelength of from about 253.7 nm.

Advantageously the blood is heated to a temperature of from about 0 to about 56°C while being contacted with the ozone gas and ultraviolet radiation. The preferred temperature ranges from 37 to 43°C and the preferred temperature is 42.5°C.

Preferably the method is employed on a 10 ml aliquot of blood. Preferably the blood is contacted with the ozone gas and ultraviolet radiation for a period of about 3 minutes.

The blood may be human blood.

The invention relates to blood treated by a method as described above and also relates to the use of blood treated by a method as described above and a method as described above in the preparation of a medicament.

The medicament may be for the treatment of peripheral vascular disease, a thrombotic disease, coronary thrombosis, pulmonary thrombosis, stroke, pre-eclampsia, and hypertension.

The invention will now be described in greater detail.

THIS PAGE IS FOLLOWED BY PAGE 7

As evidenced by the data set forth in Examples 1 and 2 below, Applicant has found that satisfactory inhibition of platelet aggregation can only be achieved when the blood is treated with a combination of ozone gas and ultraviolet radiation. Treatment of blood solely with ozone gas produces minimal inhibition of blood platelet aggregation. Moreover, treatment of blood solely with ultraviolet light produces no inhibition of platelet aggregation whatsoever.

The combined treatment with ozone gas and ultraviolet light, however, has unexpectedly been found to produce significant inhibition of blood platelet aggregation, which is useful in treating a variety of disorders associated with blood platelet aggregation.

The term "aggregation of blood platelets" as used herein refers to the sticking together of platelets to other platelets and/or to the walls of a blood vessel.

The ozone gas used in connection with the inventive method has a concentration of ozone of from about 0.5 to about 100 μg/ml. Preferably, the ozone gas has a concentration of from about 5 to about 50 μg/ml.

Ultraviolet radiation having a wavelength of about 253.7 nm has been found to provide the results of the invention, when utilized in conjunction with the ozone gas treatment. It is believed that ultraviolet radiation having emission wavelengths corresponding to standard UV-A and UV-B sources would also provide acceptable results.

The blood is preferably heated to a temperature of from about 0 to about 56 °C while being contacted with the ozone gas and ultraviolet radiation. The blood is preferably heated to about 37-43 °C, most preferably about 42.5 °C, while being contacted with the ozone gas and ultraviolet radiation.

The aliquot of blood treated by the inventive technique is withdrawn from the human patient in any conventional manner known in the art. The method preferably involves removing about 10 ml of blood, treating the same with ozone gas and ultraviolet radiation, then returning the treated blood to the patient by intramuscular injection. Other conventional techniques for readministering the blood may be employed, such as intravenous injection, subcutaneous injection, and intraperitoneal injection. The readministration of small volumes of host blood in this fashion is termed micro-auto-hemotherapy.

The invention also contemplates an embodiment wherein the blood is continuously removed from the patient's body and circulated through an apparatus which treats the blood with ozone gas and ultraviolet light before returning the blood to the patient. This procedure would have particular utility, for example, during the performance of operative procedures, such as coronary bypass surgery.

The blood is contacted with the ozone gas and ultraviolet radiation for a period of time sufficient to effectively inhibit the aggregation of blood platelets. A treatment period of from about 1 minute to about 60 minutes, and preferably about 3 minutes, has been found to provide satisfactory inhibition of platelet aggregation.

The method should be carried out under sterile conditions known to those of ordinary skill in the art.

The method of the invention may be carried out using conventional apparatus for ozonating blood and irradiating blood with ultraviolet light known to those skilled in the medical art. Preferably, an apparatus as disclosed in U.S. Patent No. 4,968,483 is employed to carry out the method of the invention. The disclosure of U.S. Patent No. 4,968,483 is incorporated herein in its entirety by reference.

In a preferred aspect of the invention, a method of inhibiting the aggregation of blood platelets in a human is provided, which comprises:

(a) removing an aliquot of blood from a human;

(b) contacting the blood with a blood platelet-inhibiting effective amount of from about 5 to about 50

μg/ml of ozone gas and ultraviolet radiation having a wavelength of about 253.7 nm, while heating the blood to a temperature of from about 37 to about 43 °C; and

(c) readministering the treated blood to the human.

The invention also contemplates a method of treating a condition in a human associated with blood platelet aggregation, which comprises:

(a) removing an aliquot of blood from a human;

(b) contacting the blood with a blood platelet-inhibiting effective amount of ozone gas and ultraviolet radiation; and

(c) readministering the treated blood to the human.

The useful and preferred ranges of ozone concentration, ultraviolet wavelength, temperature, and other parameters of the method of treatment are the same as described above with regard to the method of inhibiting blood platelet aggregation.

Those skilled in the art will appreciate that the method of inhibiting blood platelet aggregation provided by the invention will have therapeutic utility for treating a wide range of disease states associated with the aggregation of blood platelets in humans.

The term "treating" as used herein refers to the alleviation or prevention of a particular disorder. In the case of traumatic conditions such as stroke, preventative treatment is obviously preferred. Also, although the term "human" is used to describe the preferred host, those skilled in the art will appreciate that the methods of the

invention would have similar utility with other mammals.

The following diseases are illustrative of known conditions which may be associated with the aggregation of blood platelets, and which are treatable according to the inventive method: peripheral vascular disease; arterial and venus disorders including thrombotic diseases such as coronary thrombosis, pulmonary thrombosis, arterial thrombosis, and venus thrombosis; stroke; pre-eclampsia; and hypertension. This list is merely illustrative of conditions which are associated with platelet aggregation; those of ordinary skill in the art will appreciate that other disease states associated with an aggregation of blood platelets may be treated with the inventive technique.

With regard to peripheral vascular disease, the disease is thought to be associated with a reduction of endothelial-derived relaxing factor (EDGF), low levels of which lead to a contraction of the smooth muscle of blood vessels, and hence a reduction in the diameter of the lumen of the vessel and a reduction in blood flow. The major naturally occurring EDGF is nitric oxide. In addition, nitric oxide stabilizes blood platelets, reducing their aggregation. An increase in EDGF (nitric oxide) levels, therefore, has a double beneficial effect on the circulatory system: it inhibits aggregation of platelets, making the blood more fluid, and it enlarges the diameter of the vessels, improving the flow. The reverse, a reduction in nitric oxide levels, is present in peripheral vascular disease.

As illustrated in Example 2 below, the method of the

invention is believed to increase nitric oxide levels in the blood, which may explain the mode of action in the inventive treatment of peripheral vascular disease and other conditions associated with blood platelet aggregation.

Pre-eclampsia may lead to eclampsia, an acute hypertensive crisis that may occur in the second or third trimester of pregnancy. Although the precise etiology is unknown, overactive platelet activity leading to the formation of thrombi in the placenta is believed to be a cause of the condition. The inventive method, which results in a stabilization of the patient's blood platelets and an inhibition of platelet aggregation, is therefore a potential treatment modality. In particular, the method of the invention may be preferred over conventional antiplatelet therapies, where the administration of drugs to the mother is counterindicated.

The following examples are given to illustrate the invention but are not deemed to be limiting thereof. All percentages given throughout the application are percents of platelet inhibition, unless otherwise indicated.

EXAMPLE 1

Inhibition of Blood Platelet Aggregation

The following experiment was conducted to study the effects of ozone/ultraviolet light treatment on blood platelet activity.

Experimental Procedure

Samples (20 ml) of peripheral blood were taken from 10 individuals for 13 separate experiments. Each sample was

divided into two aliquots. The first aliquot was treated according to the inventive technique, as follows:

The 10 ml aliquot was treated in vitro for three minutes with ozone gas (variable ozone concentration of 5-50 μg/ml) and ultraviolet light (253.7 nm), at a temperature of 42.5°C. An apparatus as disclosed in U.S. Patent No. 4,968,483 was utilized to carry out the treatment of the blood sample.

The second 10 ml aliquot from each sample served as an untreated control.

Platelets were isolated from the control or treated samples by centrifugation, and their ability to aggregate in response to different concentrations of ADP (a natural platelet stimulator) was measured in an aggregometer. A sample of both ozone-treated and untreated blood was used for quantitation of platelet numbers, using a Coulter counter. In some of the experiments described below, aliquots of the blood were treated with different concentrations of ozone. In other experiments performed, the blood was treated in the presence and absence of UV-light irradiation.

Platelet aggregation in the ozone-treated blood was expressed as a percentage of aggregation in the same-person untreated control blood.

Resul

As shown in Table 1, the results of the experiments indicate that treatment of blood with ozone and ultraviolet light according to the invention inhibits the aggregation of

blood platelets. Furthermore, there is an indication that this inhibition is dose related to the ozone concentration (See Table 2).

The effect of high levels of ozone on ADP-stimulated blood platelets

High levels of ozone (between 35 and 50 μg/ml) caused a measurable inhibition of ADP-induced platelet aggregation (arbitrarily taken as 33.3% inhibition) in 11 of the 13 experiments (8 of the 10 individuals). Taking all the data on all 10 individuals, the mean inhibition of platelet aggregation was 49.2 +/- 27.8% (mean +/- sd). There was no significant difference between the inhibitory effects on blood taken from males and females (mean inhibition 48.1% and 50.7%, respectively).

This inhibition appears to relate to the concentration of ADP (aggregation stimulator) over the concentration range of 0.01-0.1mM ADP, with lower inhibition at higher concentration of platelet agonist. However, this relationship did not hold at higher ADP concentrations (Table 1) and could be spurious, although the level of inhibition at 0.01mM ADP is significantly greater than at 0.1mM ADP (71% vs. 95%, $p < 0.02$).

TABLE 1

The effect of high levels of ozone on the aggregation of human blood platelets in the presence of varying concentrations of ADP

Date (Individual)	Concentration of ozone (µg/ml)	Concentration of ADP (mM)	Percent Inhibition of Aggregation	Platelet Count Before Ozone	Platelet Count After Ozone
21.11.91 (F1)	50	10	100		
27.11.91 (M1)	50	5	83.3		
		10	71.4		
		30	75.0		
2.12.91 (F2)	50	10	0		
		30	10.0		
		100	27.3		
3.12.91 (M2)	50	0.5	67.1		
		1	57.1		
		5	50.0		
		30	88.1		
6.12.91 (M3)	50	0.1	0	34	49
		0.1	6.2		
		0.5	4.0		
		0.5	0		
11.12.91 (M4)	50	0.05	67.0	46	93
		0.1	62.4		
		1.0	74.3		
		10.0	50.0		
12.12.91 (M5)	50	0.01	67.0	51	121
		0.1	7.1		
		1.0	35.7		
13.12.91 (F1)	50	0.01	63.4	33	87
		0.05	22.7		
		0.1	30.4		
		0.5	15.4		
		1.0	20.8		
		5.0	20.0		
		10.0	27.6		
9.01.92 (M6)	50	0.01	34.2	34	40
		0.05	31.0		
		0.1	9.8		
		0.5	15.4		
		1.0	26.2		
		5.0	31.3		
10.01.92 (F3)	50	0.001	71.4	49	64
		0.005	37.5		

			0.01	69.8		
			0.05	33.8		
			0.1	31.2		
			0.5	10.1		
			1.0	21.8		
	13.01.92 (F4)	50	0.005	100	49	52
			0.01	100		
			0.05	95.2		
			0.1	92.9		
			0.5	95.8		
			1.0	91.6		
			5.0	95.8		
			10.0	80.0		
	15.01.92 (F1)	40	0.01	90.0	81	66
			0.05	71.4		
			0.1	40.7		
			0.5	87.0		
			1.0	81.8		
			5.0	95.5		
			10.0	85.2		
			50.0	84.0		
			100.0	79.1		
	21.01.92 (M2)	35	0.01	67.1	68	79

The following is a summary of the data set forth in Table 1:

ADP mM	0.01	0.05	0.10	0.50	1.00	5.00	10.0
% inhibition of aggregation	70.8 +/-20.9	53.5 +/-26.1	34.7 +/-28.4	37.6 +/-38.4	50.3 +/-28.7	60.7 +/-35.2	60.7 +/-30.4
N=	6	6	8	7	7	4	4

The effect of high levels of ozone on total whole blood platelet counts

As any apparent reduction in platelet aggregation following ozone treatment of whole blood could be caused by a loss of platelets from the blood during treatment, total whole platelet counts were performed on the treated and untreated whole blood samples in 9 experiments on blood from 8 individuals. Overall, the platelet count was 115.5 +\- 59.8% of the untreated level following ozonization (range 82-264%).

Thus, the total platelet counts before and after ozone/UV treatment do not indicate a major loss of platelets from the blood as a result of ozonization.

The effect of different concentrations of ozone on the inhibition of aggregation of human blood platelets stimulated with ADP

Three different concentrations of ozone (5, 25, and 50 µg/ml) were used at a range of ADP concentrations in 4 experiments on 4 different individuals. Bulking the data for different ozone concentrations from each individual and calculating the mean for the data from the 4 experiments

indicated that there was some dose response relationship between the concentration of ozone used and the inhibition of platelet aggregation (See Table 2). Although overall these differences were not significant, in two of the four individuals there was a significantly greater inhibitory effect of ozone at 50 μg/ml then at 5 μg/ml (See Table 3).

TABLE 2

The effect of different concentrations of ozone on inhibition of platelet aggregation in the presence of ADP

Date (Individual)	Concentration of ozone (μg/ml)	Concentration of ADP (mM)	Percent Inhibition of Aggregation	Platelet Count Before Ozone - After Ozone
3.12.91 (M2)	5	0.1	27.3	
	25	0.1	100	
	5	0.5	0	
	25	0.5		
	50	0.5	67.1	
	5	1.0	0	
	25	1.0	28.6	
	50	1.0	57.1	
	5	5.0	0	
	25	5.0	25.0	
	50	5.0	50.0	
	5	30.0	50.0	
	25	30.0	62.0	
	50	30.0	88.1	
9.01.92 (M6)	5	0.01	20.1	34 43
	25	0.01	28.9	45
	50	0.01	34.2	40
	5	0.05	0	
	25	0.05	5.2	
	50	0.05	31.0	
	5	0.1	9.8	
	25	0.1	1.4	
	50	0.1	9.8	
	5	0.5	0	
	25	0.5	0	
	50	0.5	15.4	

		5	1.0	22.5		
		25	1.0	13.7		
		50	1.0	26.2		
		5	5.0	0		
		25	5.0	17.8		
		50	5.0	31.5		
	10.01.92	5	0.001	57.1	49	73
	(F3)	25	0.001	85.7		90
		50	0.001	71.4		64
		5	0.005	37.5		
		25	0.005	80.0		
		50	0.005	37.5		
		5	0.01	66.4		
		25	0.01	83.2		
		50	0.01	69.8		
		5	0.05	44.9		
		25	0.05	66.9		
		50	0.05	33.8		
		5	0.1	29.3		
		25	0.1	61.0		
		50	0.1	31.2		
		5	0.5	39.4		
		25	0.5	54.5		
		50	0.5	10.1		
		5	1.0	21.8		
		25	1.0	52.9		
		50	1.0	21.8		
	13.01.92	5	0.005	100	49	60
	(F4)	25	0.005	100		85
		50	0.005	100		52
		5	0.01	100		
		25	0.01	87.5		
		50	0.01	100		
		5	0.05	84.8		
		25	0.05	97.1		
		50	0.05	95.2		
		5	0.1	82.9		
		25	0.1	91.4		
		50	0.1	92.9		
		5	0.5	83.3		
		25	0.5	95.8		
		50	0.5	95.8		
		5	1.0	83.2		
		25	1.0	89.5		
		50	1.0	91.6		

5	5.0	79.2
25	5.0	91.7
50	5.0	95.8
5	10.0	85.3
25	10.0	80.0
50	10.0	80.0

The following is a summary of the data set forth in Table 2:

Concentration of ozone (μg/ml)	5	25	50
Platelet aggregation (%) (mean +/- sd, n=4)	38.5+/-30.9	56.5+/-29.4	55.9+/-26.4

TABLE 3

The effect of different concentrations of ozone on inhibition of platelet aggregation in two individuals

Concentration of ozone (μg/ml)	5	25	50
Platelet aggregation M2 (%)	15.5+/-20.2	53.9+/-30.0	65.6+/-14.4
Difference from 5 μg/ml		ns	p<0.01
Platelet aggregation M6 (%)	8.7+/-9.6	11.2+/-10.2	24.7+/-9.0
Difference from 5 μg/ml		ns	p<0.02

ns=not significant

The effect of UV light on the response of platelets to ozone

The effect of ozone on the aggregation of human blood platelets was investigated at different concentrations of ADP, in the presence or absence of UV light. The results, shown in Table 4, indicate that, although there may be some platelet aggregation-inhibitory response to ozone alone, this is nearly always greater in the presence of UV light and the effect of UV light was highly significant ($p<0.001$) in this single experiment. This result was also repeated in a second experiment, using a single concentration of ADP

(0.01 mM). The results of this second experiment are set forth in Table 5.

TABLE 4

The effect of UV light on the inhibition of ADP-induced platelet aggregation by ozone at a concentration of 40 µg/ml. (Experiment date 15.01.92, individual F1)

Concentration ADP (mM)	Inhibition of platelet aggregation (%)	
	+UV	-UV
0.01	90.0	60.0
0.05	71.4	0
0.1	40.7	40.7
0.5	87.0	0
1.0	81.8	0
5.0	95.5	19.4
10.0	85.2	18.5
50.0	84.0	16.0
100.0	79.1	4.2
Mean +/- sd	79.4+/-15.1	17.6+/-19.6 ($p<0.001$)

TABLE 5

The effect of UV light on platelet aggregation induced by ADP (0.01 mM) in the presence or absence of ozone. (Experiment date 21.01.92, individual M2)

Percent inhibition of platelet aggregation		
Ozone 35 µg/ml + UV	Ozone 35 µg/ml - UV	No ozone, UV alone
83.4%	11.2%	0%

In summary, the results of Example 1 indicate that the in vitro treatment of an aliquot of blood with ozone gas and ultraviolet light inhibits the aggregation of blood

platelets. This platelet inhibition has been found to be dose related to the ozone concentration. Further, platelet inhibition was found to critically depend on the combined treatment of ultraviolet light and ozone gas, as evidenced in Tables 4 and 5. Treatment with ozone gas alone resulted in minimal inhibition of platelet aggregation, while treatment with ultraviolet light alone produced no inhibition of platelet aggregation.

EXAMPLE 2

Measurement of Nitric Oxide

In order to elucidate the mechanism whereby ozonization/ UV light affects the aggregation of platelets in treated blood, the concentration of certain oxidized forms of nitrogen were measured.

The direct measurement of nitric oxide is difficult to achieve. However, nitric oxide is an intermediate in a metabolic pathway in which arginine is converted to citrulline. Other stable end-products are nitrates and nitrites.

Accordingly, the nitric oxide content for several samples of blood treated with ultraviolet light and ozone gas according to Example 1 were indirectly determined by measuring the combined nitrate plus nitrite concentrations in the samples before and after treatment with ozone/UV light, after converting nitrite to nitrate.

The results show that there is a small increase in nitrate plus nitrite concentrations after treatment

according to the invention. This increase was consistently found in samples treated with ozone gas/UV light. Thus, nitric oxide levels may be enhanced by the treatment with ozone gas/UV light, and this may be part of the mode of action by which an inhibition of blood platelet aggregation is achieved by the invention. This therapeutic effect would be consistent with the etiology of peripheral vascular disease described above.

Conclusions

The data of Examples 1 and 2 suggest that the treatment of blood with ozone gas and ultraviolet light according to the invention is actually inducing an inhibition of platelet aggregation for the following reasons:

1. The inhibitory effect is at least partially dependent on the concentration of ADP, ozone being more inhibitory at lower ADP concentrations. This may be interpreted as the higher agonist concentrations partially overcoming the inhibitory effect of ozone by "hyperstimulating" the platelets. This suggests that the inhibition is at least partially reversible, and is probably not acting by destroying the platelet's ability to aggregate.

2. The inhibitory effect appears to be dose related to ozone concentration, with higher concentrations of ozone resulting in a greater inhibition of platelet aggregation.

3. The inhibitory effect is UV-dependent, suggesting that this is not a non-specific toxic effect caused by the oxidative capacity of the ozone gas.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications are intended to be included within the scope of the following claims.

CLAIMS:

1. A method of treating blood which comprises contacting the blood with a blood platelet-aggregation inhibiting effective amount of ozone gas and ultraviolet radiation.

2. The method of Claim 1 wherein the ozone gas has a concentration of from about 0.5 to about 100 μg/ml.

3. The method of Claim 2, wherein the ozone gas has a concentration of from about 5 to about 50 μg/ml.

4. The method of any one of the preceding Claim wherein the ultraviolet radiation has a wavelength of from about 253.7 nm.

5. The method of any one of the preceding Claims wherein the blood is heated to a temperature of from about 0 to about 56^{0}C while being contacted with the ozone gas and ultraviolet radiation.

6. The method of Claim 5, wherein the blood is heated to a temperature of from about 37 to about 43^{0}C while being contacted with the ozone gas and ultraviolet radiation.

7. The method of Claim 6, wherein the blood is heated to a temperature of about 42.5^{0}C while being contacted with the ozone gas and ultraviolet radiation.

8. The method of any one of the preceding Claims wherein the method is performed on an aliquot of blood of about 10 ml of blood.

9. The method of any one of the preceding Claims wherein the blood is contacted with the ozone gas and ultraviolet radiation for a period of about 3 minutes.

10. The method of any one of the preceding Claims wherein the blood is human blood.

11. Blood treated by a method of any one of Claims 1 to 10.

12. The use of blood according to Claim 11 in the preparation of a medicament.

13. The use of a method according to any one of Claims 1 to 10 in the preparation of a medicament.

14. Use according to Claim 12 or 13 wherein the medicament is for the treatment of peripheral vascular disease.

15. Use according to Claim 12 or 13 wherein the medicament is for the treatment of a thrombotic disease.

16. Use according to Claim 12 or 13 wherein the medicament is for the treatment of coronary thrombosis.

17. Use according to Claim 12 or 13 wherein the medicament is for the treatment of pulmonary thrombosis.

18. Use according to Claim 12 or 13 wherein the medicament is for the treatment of a stroke.

19. Use according to Claim 12 or 13 wherein the medicament is for the treatment of pre-eclampsia.

20. Use according to Claim 12 or 13 wherein the medicament is for the treatment of hypertension.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 93/00258

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61M1/36; A61K35/14; A61K41/00

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61M ; A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	PATENT ABSTRACTS OF JAPAN vol. 8, no. 34 (C-210)15 February 1984 & JP,A,58 198 466 (TEIJIN KK) 18 November 1983 see abstract	1
A	DE,A,2 926 523 (STADTLAENDER) 22 January 1981 see page 2, line 18 - line 22 see page 5, line 32 - page 6, line 16; claim 1; figures	1
A	US,A,4 968 483 (MÜLLER ET AL.) 6 November 1990 cited in the application see abstract; figures see column 4, line 12 - line 27; claims 8,17	1

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 MAY 1993	19. 05. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	ZEINSTRA H.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A,P	DATABASE WPIL Week 9307, Derwent Publications Ltd., London, GB; AN 93-058408 & US,A,7 764 906 (US DEPT HEALTH & HUMAN SERVICE) 15 December 1992 see abstract	1
A	US,A,3 325 641 (JONES) 13 June 1967 see column 1, line 15 - line 35; figures	1

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

GB 9300258
SA 70190

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 06/05/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE-A-2926523	22-01-81	None	
US-A-4968483	06-11-90	DE-U- 8704467	26-05-88
		AU-B- 613333	01-08-91
		AU-A- 1000288	28-07-88
		GB-A,B 2242367	02-10-91
US-A-3325641		None	

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82